American Journal of Pathology, Vol. 159, No. 6, December 2001

Immunohistochemical Detection of Myeloperoxidase and Its Oxidation Products in Kupffer Cells of Human Liver

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Oxidative damage to tissue proteins has been implicated in the pathogenesis of liver disease, but the mechanisms that promote oxidation *in vivo* are unclear. Hydrogen peroxide is transformed into an array of potentially damaging reactants by the heme protein myeloperoxidase. This proinflammatory enzyme is expressed by circulating neutrophils and monocytes but is generally thought to be absent from tissue macrophages. To determine whether myeloperoxidase is present in Kupffer cells, the fixed-tissue macrophages of liver, Western blot analysis, and immunohistochemistry were performed. Two different antibodies monospecific for myeloperoxidase identified a 60-kd protein, the predicted molecular mass of myeloperoxidase, in human liver extracts. Immunostaining detected the enzyme in sinusoidal lining cells of normal and diseased human livers. Immunofluorescence confocal microscopy demonstrated co-localization of myeloperoxidase and CD68, a monocyte/macrophage marker, in sinusoidal lining cells. Numerous myeloperoxidase-expressing cells were also evident in the fibrous septa of cirrhotic livers. Immunostaining with an antibody to proteins modified by hypochlorous acid, a characteristic product of the enzyme, indicated that myeloperoxidase is enzymatically active in cases of acute liver injury and cirrhosis. These findings identify myeloperoxidase as a component of human Kupffer cells. Oxidative damage resulting from the action of myeloperoxidase may contribute to acute liver injury and hepatic fibrogenesis. *(Am J Pathol 2001, 159:2081–2088)*

Reactive intermediates generated by activated phagocytes damage biomolecules and have been implicated in the pathogenesis of various conditions including rheumatoid arthritis, atherosclerosis, malignancy, and aging.[1–6] The pathway for oxidant generation by neutrophils, monocytes, and macrophages begins with a membrane-associated NADPH oxidase that produces superoxide, which then dismutates to hydrogen peroxide (H_2O_2).[3] Neutrophils and monocytes also secrete a heme protein, myeloperoxidase, which uses the oxidizing potential of H_2O_2 to convert chloride ion into hypochlorous acid (HOCl).[3] A potent bactericidal agent, HOCl is a critical component of host defenses against invading bacteria, fungi, and viruses.[4] It has been proposed that HOCl and other oxidizing intermediates generated by myeloperoxidase also contribute to tissue damage at sites of inflammation.[5,6]

In vivo and *in vitro* studies of myeloid cells indicate that myeloperoxidase is synthesized at the promyelocytic stage of differentiation.[4,7] The enzyme represents ~5% of neutrophil and ~1% of monocyte protein but has long been believed to be absent from macrophages.[8] Indeed, myeloperoxidase protein and mRNA rapidly disappear from freshly isolated human monocytes as they differentiate into macrophages *in vitro*.[9] In contrast, recent studies suggest that myeloperoxidase is present in tissue macrophages of human atherosclerotic lesions. Enzymatically active myeloperoxidase has been extracted from this macrophage-rich atherosclerotic tissue and immunohistochemical studies have shown co-localization of myeloperoxidase and macrophages in atherosclerotic lesions.[10,11] Moreover, elevated levels of 3-chlorotyrosine, a specific product of myeloperoxidase at plasma concentrations of halide, have been detected in atherosclerotic tissue and in low-density lipoprotein isolated from these vascular lesions.[12] Immunohistochemical studies have also demonstrated HOCl-modified proteins in these lesions.[13] Collectively, these data strongly support the hypothesis that myeloperoxidase is present in atherosclerotic lesions and is catalytically active *in vivo*. These observations raise the intriguing possibility that macrophages express myeloperoxidase under certain conditions *in vivo* and that oxidants generated by macrophage-

Supported in part by the Medical Research Service of the Veterans Administration (to K. E. B.) and the National Institutes of Health (AG15012, HL64344, DK56341 to J. W. H.), and the Washington University-Pharmacia-Monsanto-Searle Research Program.

Accepted for publication August 17, 2001.

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associated myeloperoxidase may participate in the pathogenesis of inflammatory diseases.

Myeloperoxidase may contribute to tissue injury by several mechanisms. HOCl is a potent oxidant that attacks nucleophilic amino groups, generating reactive aldehydes and chloramines.[14,15] It also reacts with unsaturated lipids to form chlorohydrins.[16] Lipid peroxidation and protein cross-linking can be catalyzed by tyrosyl radical, which results from the oxidation of tyrosine by myeloperoxidase.[17,18] In addition, HOCl has been shown to inactivate the protease inhibitor α-1-antitrypsin[19] and to activate latent neutrophil collagenase.[20] Conversely, myeloperoxidase has inhibitory effects on lymphocyte function[21] and can suppress inflammation by inactivating soluble chemotactic factors such as C5a.[22] Thus, in addition to being a direct cause of tissue injury, myeloperoxidase may modulate aspects of the inflammatory response.

Kupffer cells, the resident macrophages of the liver, comprise 80 to 90% of the body's fixed tissue macrophage population.[23] Because these cells seem to be involved in the pathogenesis of a variety of liver diseases and because myeloperoxidase has been detected in the macrophage foam cells of atheromatous lesions, we hypothesized that Kupffer cells might be a source of myeloperoxidase. We report here that two monospecific rabbit polyclonal antibodies to myeloperoxidase recognized a protein in detergent extracts of human liver tissue that co-migrated with myeloperoxidase on Western blotting. Myeloperoxidase was also detected immunohistochemically in the Kupffer cells of both nondiseased and diseased human livers. These observations indicate that myeloperoxidase is present in human Kupffer cells and raise the possibility that the enzyme may be an important source of oxidative damage during liver injury.

Materials and Methods

Rabbit polyclonal antibody against human myeloperoxidase (A398) and monoclonal mouse anti-human macrophage CD68 (M814) were purchased from DAKO Corp. (Carpinteria, CA). Rabbit polyclonal anti-myeloperoxidase (K50891R) was purchased from Biodesign International (Kennebunk, ME). HOP-1, a mouse monoclonal antibody raised against HOCl-modified low-density lipoprotein (LDL), was obtained from Dr. Ernst Malle (Karl-Franzens University, Graz, Austria). This antibody has been previously shown to react with HOCl-modified proteins including bovine serum albumin, human serum albumin, low-density lipoprotein, and high-density lipoprotein, but not with native low-density lipoprotein, or low-density lipoprotein-modified by Cu^{2+} oxidation, malondialdehyde (MDA), or 4-hydroxy-2-nonenal (HNE).[24]

Liver Tissues

Fresh human liver was obtained at the time of liver transplantation for end-stage or fulminant disease or resection of mass lesions and was immediately stored at −70°C. Archived blocks of normal and diseased human tissues were obtained from the Department of Pathology, Saint Louis University Health Sciences Center. This study was approved by the Institutional Review Board of Saint Louis University.

Fresh mouse liver was obtained from myeloperoxidase-deficient and wild-type animals.[25] The animals were perfused with 10 ml of ice-cold phosphate-buffered saline and the livers rapidly removed, fixed in formalin, and embedded in paraffin. The Animal Studies Committee of Washington University School of Medicine approved all animal studies.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis and Immunoblotting

Liver tissue was homogenized in 20 volumes of Tris buffer (50 mmol/L, pH 6.8) containing 5% (v/v) β-mercaptoethanol and 1% (w/v) sodium dodecyl sulfate. A 1-ml aliquot of the homogenate was boiled for 5 minutes, sonicated three times for 10 seconds, and spun at 10,000 × *g* for 10 minutes. The protein content of the supernatant was quantitated using a modification of the Lowry assay and the indicated quantities loaded into the wells. Proteins were subjected to electrophoresis on 10 to 20% (w/v) gradient polyacrylamide gels (Mini Protean II system; BioRad Laboratories, Richmond, CA). The resolved proteins were electrophorectrically transferred to polyvinylidene difluoride membranes (MSI, Westboro, MA). Nonspecific binding sites were blocked with 20% nonfat dried milk/0.5% Tween-Tris-buffered saline. After thorough washing, the membrane was first incubated with rabbit anti-human myeloperoxidase antibody diluted 1:10,000 then with peroxidase-conjugated goat anti-rabbit IgG diluted 1:10,000 (Sigma, St. Louis, MO). After extensive washing, bound antibody was detected using chemiluminescence according to the manufacturer's instructions (ECL; Amersham International, Little Chalfont, Bucks, UK).

Immunoaffinity Purification of Myeloperoxidase Antibody

Electrophoresis was performed as above using genuine myeloperoxidase (500 ng/well). The myeloperoxidase used was apparently pure as determined by spectroscopy (R/Z = 0.81) and by electrophoresis under denaturing and nondenaturing conditions followed by Coomassie blue staining and assessment of peroxidase activity, respectively.[26] After transfer to the polyvinylidene difluoride membrane and incubation with the DAKO anti-myeloperoxidase antibody, a strip was cut from one side of the membrane and processed as indicated above to localize the antigen-antibody complex. This strip was aligned with the remaining membrane, the area containing the antigen and primary antibody excised, and antibody bound to myeloperoxidase eluted in 100 mmol/L of glycine buffer (pH 2.5) for 10 minutes and neutralized with 1 mol/L of Tris (pH 8.0).[27]

Immunohistochemistry

Tissue sections (4- to 5-μm thick) were cut from formalin-fixed, paraffin-embedded livers. Sections were deparaffinized and rehydrated by passage through a graded series of ethanol and distilled water. For myeloperoxidase immunohistochemistry, the antigen was retrieved by heating the slides in a pressure cooker in Tris-buffered saline with 0.075% Tween-20 (pH 7.6) for 10 minutes.[28] Endogenous peroxidase activity was quenched by incubation in 0.3% v/v H_2O_2 in methanol for 20 minutes at room temperature.[29] Sections were incubated at room temperature for 30 minutes with polyclonal rabbit anti-human myeloperoxidase antibody diluted 1:1500. Staining of murine liver sections was performed identically, with the exception that the polyclonal rabbit anti-human myeloperoxidase antibody was diluted 1:150. Sections for CD68 and HOP-1 were handled in a similar manner except for the omission of the antigen-retrieval step. The CD68 antibody was used at a dilution of 1:250 and HOP-1 antibody at 1:500 with incubation times of 1 and 2 hours, respectively. Biotinylated secondary antibodies were used at a 1:200 dilution. Immunostaining was performed using an avidin-biotin-horseradish peroxidase system (Vector Laboratories, Burlingame, CA) with 3-amino-9-ethylcarbazole as the chromogen for myeloperoxidase, diaminobenzidine for CD68, and True Blue (Kirkegaard and Perry Laboratories, Gaithersburg, MD) for HOP-1. Primary antibodies were omitted on sections serving as negative controls. Sections were counterstained and those stained with 3-amino-9-ethylcarbazole were mounted in aqueous mounting medium before application of coverslips. For immunofluorescence, liver sections were deparaffinized, rehydrated, and antigen-retrieved as above before incubation with a mixture of the myeloperoxidase and CD68 antibodies for 2 hours. The sections were subsequently incubated with fluorescein isothiocyanate-labeled anti-mouse IgG and Texas Red-labeled anti-rabbit IgG antibodies (Vector Laboratories) at a dilution of 1:50 and examined using confocal microscopy.

Results

Myeloperoxidase is a dimeric enzyme composed of two heavy subunits (~60 kd) and two light subunits (~15 kd).[30] To assess the specificity of the two different rabbit polyclonal myeloperoxidase antibodies (DAKO and Biodesign) used for our studies, purified myeloperoxidase was subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis and Western blotting. A major band and a minor band with apparent molecular masses of 60 kd and 15 kd, respectively, were recognized by both antibodies (Figure 1, lane 1). Minor bands were also observed at 40 kd and 20 kd, consistent with autocatalytic products of myeloperoxidase previously reported.[31] In extracts of liver obtained from patients undergoing transplantation or liver resection for a variety of liver diseases, both antibodies recognized a single 60-kd protein that co-migrated with authentic myeloperoxidase (Figure 1, lanes 2 to 6). Immunoaffinity-purified myeloperoxidase antibody also reacted with a protein in liver tissue that co-migrated with the heavy chain of myeloperoxidase (data not shown). A 15-kd band that co-migrated with the light chain of myeloperoxidase was observed in liver homogenates on immunoblots in which large concentrations of protein were loaded and higher concentrations of primary antibody were used (data not shown). Thus, the antibody was monospecific for myeloperoxidase in these tissues. All five liver samples examined by Western blotting contained immunoreactive myeloperoxidase (Figure 1).

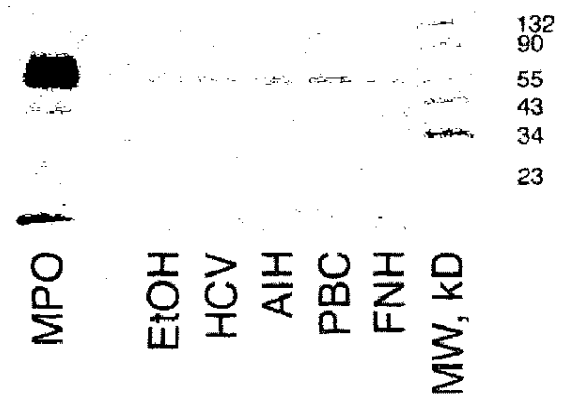

Figure 1. Immunodetection of myeloperoxidase in human livers. Purified myeloperoxidase (250 ng) and detergent extracts of liver homogenates (15 μg protein) were separated in 7% sodium dodecyl sulfate on a 10 to 20% polyacrylamide gradient gel and transferred onto a polyvinylidene difluoride membrane. Immunoreactive material was detected with a polyclonal rabbit anti-human myeloperoxidase antibody (DAKO) as described in Materials and Methods. MPO, purified myeloperoxidase; EtOH, alcoholic liver disease; HCV, chronic hepatitis C; AIH, autoimmune hepatitis; PBC, primary biliary cirrhosis; FNH, focal nodular hyperplasia.

Immunohistochemistry of nondiseased liver revealed a distinctive pattern of sinusoidal lining cell myeloperoxidase immunoreactivity (Figure 2A). In contrast, hepatocytes showed negligible immunoreactivity. Similar results were obtained with two different antibodies to myeloperoxidase (data not shown). Immunoreactivity was absent in sections exposed to primary antibody that had been preincubated with myeloperoxidase (Figure 2B). Furthermore, no immunoreactivity was observed in the livers of mice deficient in myeloperoxidase, whereas livers from wild-type mice demonstrated sinusoidal lining cell immunoreactivity similar to that seen in human livers (Figure 3). Collectively, these observations indicate that the antibodies used for the immunohistochemical studies are monospecific for myeloperoxidase and that sinusoidal lining cells of both human and murine livers reacted with the antibodies.

Some of the immunoreactive sinusoidal lining cells were slender and conformed to the contour of the sinusoidal lumen whereas others had enlarged nuclei and protruded into the sinusoidal lumen (Figure 2C). Morphologically, these cells resembled quiescent and activated Kupffer cells, respectively. A similar pattern of sinusoidal lining cell staining with the myeloperoxidase antibody was observed in sections from livers with a variety of

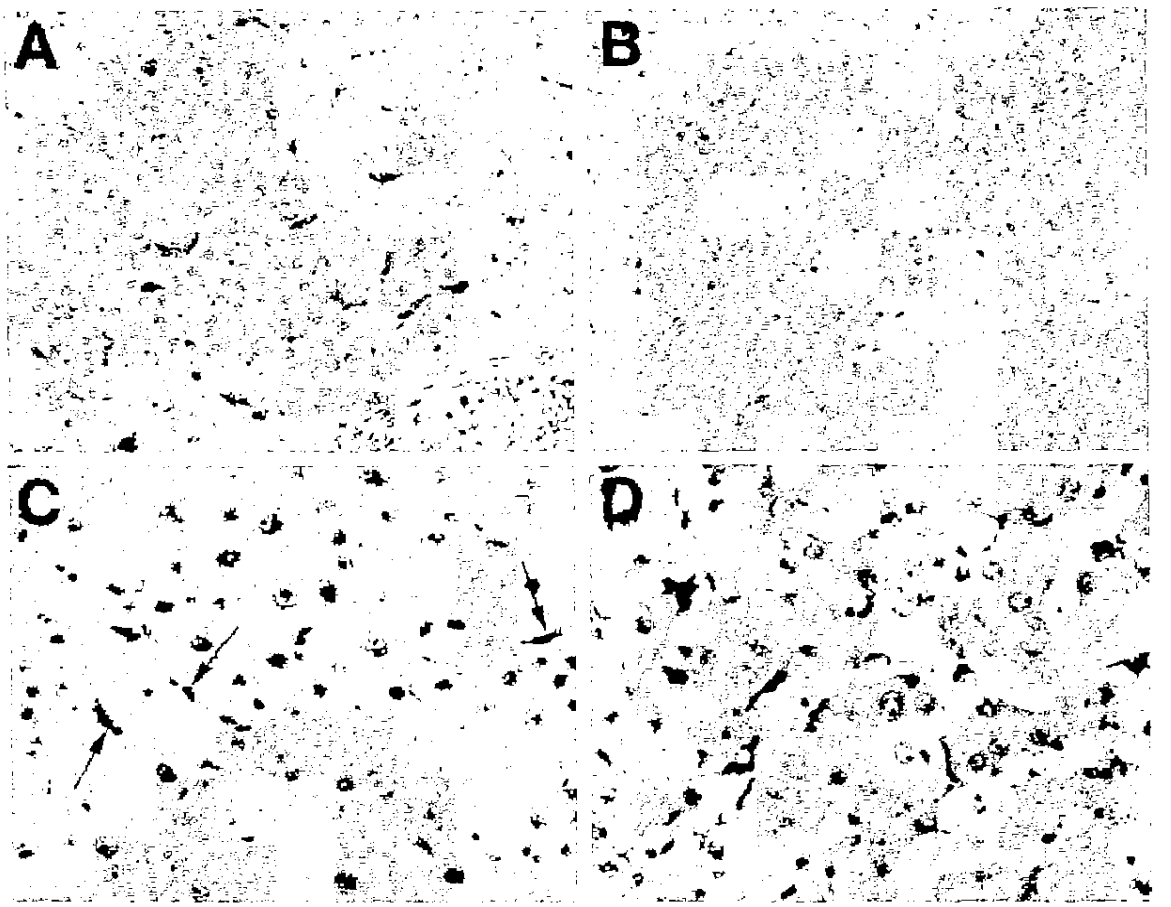

Figure 2. Myeloperoxidase-containing sinusoidal lining cells in human liver show similar morphology and localization as cells that express CD68. **A:** Section from human liver incubated with a polyclonal rabbit antibody to myeloperoxidase (DAKO). Primary antibody was detected as reaction product using an avidin-biotin-peroxidase system as described in Materials and Methods. **B:** Control section incubated with polyclonal rabbit antibody preabsorbed with myeloperoxidase. **C:** Higher power view highlighting the morphology of myeloperoxidase-positive sinusoidal lining cells (**arrows**). **D:** Kupffer cells demonstrated by staining with a monoclonal antibody to the macrophage marker CD68 and detected as black reaction product. Sections were counterstained with hematoxylin. Original magnifications: ×20 (**A** and **B**); ×40 (**C** and **D**).

acute and chronic liver diseases. Although the number of immunoreactive sinusoidal lining cells varied among the samples, there was no obvious difference between nondiseased and diseased liver nor was there an apparent lobular distribution gradient. Occasional cells within sinusoidal lumina were reactive with the antibody (data not shown) but their multilobed nuclei identified them as polymorphonuclear leukocytes and distinguished these cells from the immunoreactive cells lining the sinusoids.

To further characterize the sinusoidal lining cells that were reactive with the myeloperoxidase antibody, an antibody that recognizes CD68, an epitope specific for monocytes and macrophages,[32] was applied to serial sections of the samples. Abundant CD68-immunoreactive cells were observed lining the sinusoids of both nondiseased and diseased livers (Figure 2D). CD68-positive cells were morphologically identical to the sinusoidal lining cells that expressed myeloperoxidase, but were more numerous in all specimens examined. Based on the morphology and spatial correspondence with CD68-positive cells, the myeloperoxidase-expressing sinusoidal lining cells seemed to represent a distinct subpopulation of Kupffer cells. Double-immunofluorescence confocal microscopy with the rabbit antibody to myeloperoxidase and the murine antibody to CD68 confirmed co-localization of the enzyme and CD68 in cells lining the hepatic sinusoids (Figure 4).

In addition to sinusoidal lining cells, a second pattern of myeloperoxidase immunoreactivity was observed in samples from cirrhotic livers. Connective tissue septa contained large numbers of spindle-shaped cells that stained with the myeloperoxidase antibody (Figure 5, A and C). Spindle cell immunoreactivity was frequently accentuated in areas of bile duct proliferation. The myeloperoxidase-positive spindle cells in the septa also expressed CD68 (Figure 5B). These observations suggest that hepatic fibrosis is associated with increased numbers of myeloperoxidase-expressing macrophages in connective tissue septa.

At plasma concentrations of halide ion, myeloperoxidase is the only human enzyme known to generate the potent cytotoxic oxidant HOCl.[33,34] To determine whether

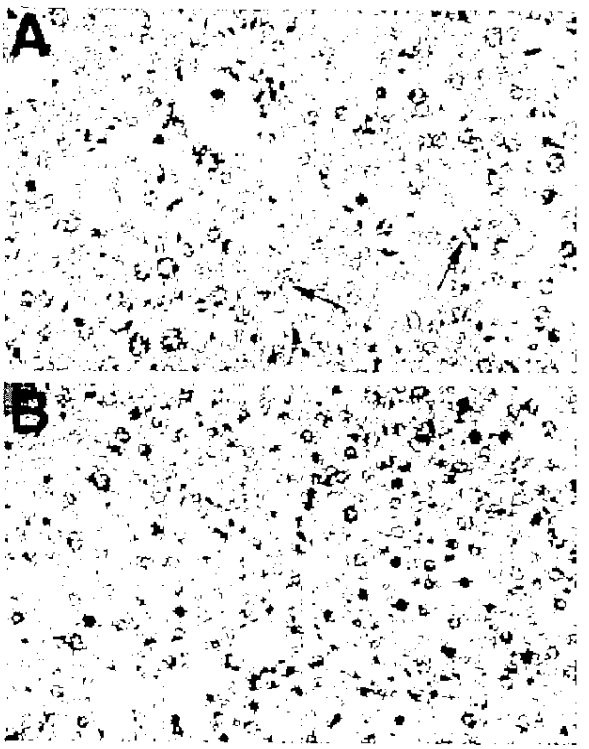

Figure [illegible] not myeloperoxidase-deficient mice are immunoreactive with an antibody to myeloperoxidase. **A:** Liver [illegible] a variety mouse incubated with polyclonal rabbit antibody to human myeloperoxidase (DAKO). Primary antibody was detected as a red reaction product using an avidin-biotin-peroxidase system as described in Materials and Methods. Myeloperoxidase-positive sinusoidal lining cells are indicated by the **arrows**. **B:** Immunostaining of the liver of a myeloperoxidase-deficient mouse. Original magnification, ×40.

myeloperoxidase is enzymatically active in human liver, we immunostained sections with HOP-1, an antibody that specifically recognizes proteins modified by HOCl.[13,24] In control livers, only occasional periportal or perivenular hepatocytes showed reactivity with HOP-1. In contrast, HOP-1 immunoreactivity was prominent in cases of massive or submassive necrosis with HOP-1-positive cells consistently observed in areas of collapse (data not shown). Interestingly, periseptal hepatocytes were also reactive with HOP-1 in some cases of cirrhosis (Figure 5D). The presence of HOCl-modified proteins in these hepatocytes was associated with the presence of myeloperoxidase-expressing cells in the septa (Figure 5, A and C). These observations indicate that HOCl-modified proteins may be present in both hepatocytes and Kupffer cells of diseased human liver.

Discussion

In this study, we present several lines of evidence that indicate that myeloperoxidase is present in Kupffer cells of human liver. First, an ~60-kd protein reactive with two different rabbit polyclonal antibody monospecific for myeloperoxidase, was present in detergent extracts of human liver. The apparent molecular mass of this protein

Figure 4. Double-immunofluorescence confocal microscopy demonstrates co-localization of myeloperoxidase and CD68 in human liver sinusoidal lining cells (yellow cells indicated by **arrows**). Numerous CD68-positive cells (green) are seen along the sinusoids (**arrowheads**), whereas a cell expressing only myeloperoxidase (red), presumably a neutrophil or monocyte, is seen within a sinusoidal lumen (**asterisk**). Sections were incubated with rabbit polyclonal antibody to myeloperoxidase (DAKO) and monoclonal antibody to CD68, then labeled with fluorescent-labeled secondary antibodies. Original magnification, ×60.

was indistinguishable from that of the heavy chain of genuine myeloperoxidase. We also detected an immunoreactive protein that migrated with the light chain of myeloperoxidase when large amounts of liver protein were studied. Second, both monospecific antibodies recognized hepatic sinusoidal lining cells that seemed to be Kupffer cells by morphological criteria. Immunoreactivity was blocked when the antibodies were preincubated with myeloperoxidase and was absent in the livers of myeloperoxidase-deficient mice. The human cells also reacted with a monoclonal antibody to CD68, a specific marker for monocytes and macrophages. Third, double-immunofluorescence confocal microscopy demonstrated co-localization of the myeloperoxidase antibody and the CD68 antibody in cells lining the hepatic sinusoid. Collectively, these observations provide strong evidence that myeloperoxidase is present in Kupffer cells, the body's largest population of fixed tissue macrophages.

Previous investigations have suggested that Kupffer cells lack myeloperoxidase. For example, Wang and colleagues[35] failed to detect HOCl after stimulation of rat Kupffer cells with phorbol ester, a treatment that stimulated rat neutrophils to produce substantial amounts of HOCl. In preliminary experiments, we observed far fewer myeloperoxidase-positive sinusoidal lining cells in rat liver than in human liver (KEB and EMB, unpublished observation). Given this difference, the inability to detect HOCl production by rat Kupffer cells by biochemical means is not surprising. In contrast, we observed significant numbers of myeloperoxidase-expressing sinusoidal

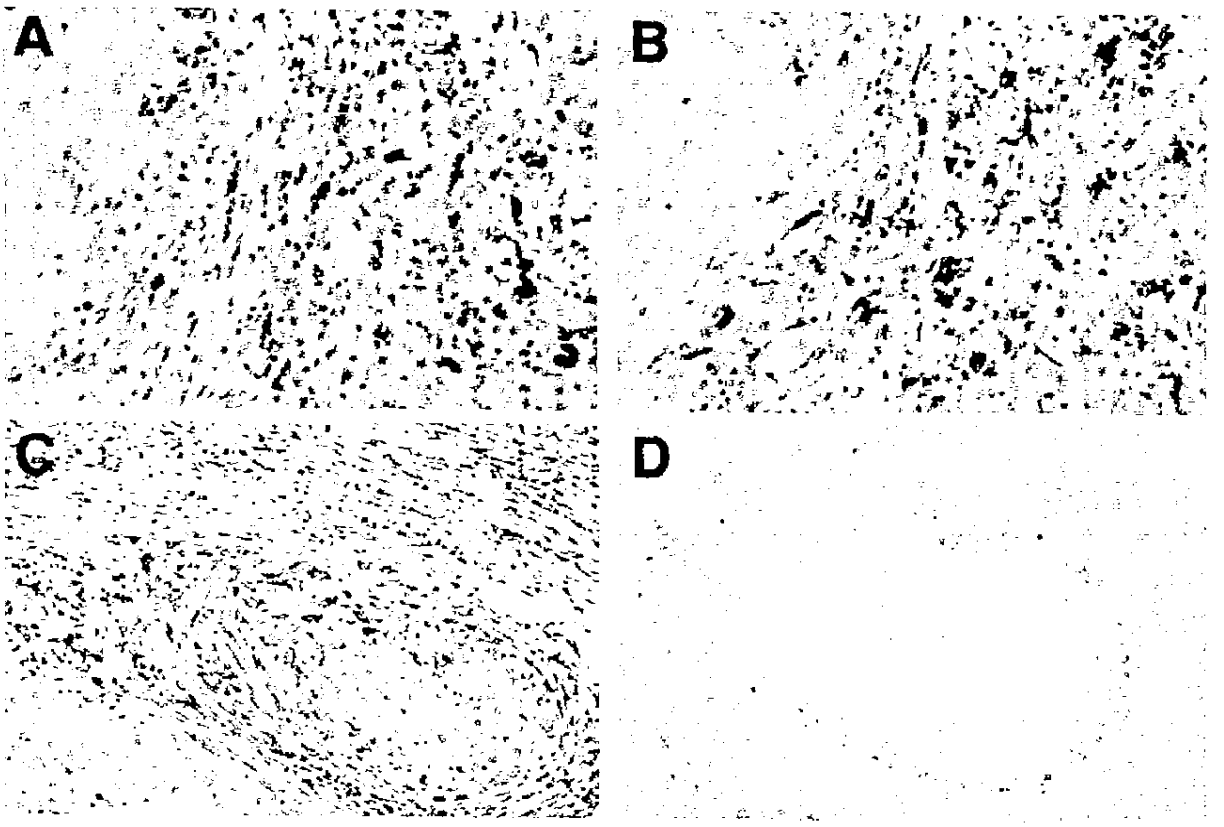

Figure 5. Large numbers of cells expressing myeloperoxidase and CD68 are observed in the septa of cirrhotic livers and are associated with the presence of hypochlorous acid-modified proteins in periseptal hepatocytes. **A:** Sections from cirrhotic human livers were incubated with a polyclonal antibody to myeloperoxidase (DAKO) and the primary antibody was detected as a red reaction product using an avidin-biotin peroxidase system as described in Materials and Methods. **B:** Serial sections from the same livers were stained for CD68 using a monoclonal antibody and diaminobenzidine as the chromogen (black reaction product). **A** and **B** were counterstained with hematoxylin. **C:** Lower power view demonstrating the presence of abundant MPO-containing cells in the septum surrounding a regenerative nodule. Staining as in **A** above. **D:** Cirrhotic liver demonstrating immunoreactivity with HOP-1, a monoclonal antibody that specifically recognizes HOCl-modified proteins, in hepatocytes of a regenerative nodule adjacent to the fibrous septum. Sections were incubated with HOP-1 as described in Materials and Methods using True Blue as the chromogen and were counterstained with Contrast Red. Original magnifications: ×40 (**A** and **B**); ×20 (**C** and **D**).

lining cells in mouse and gerbil livers, suggesting significant differences among species (KEB and EMB, unpublished observation). Similar observations have been made for monocytes. For example, myeloperoxidase represents ~1% of human monocyte protein but is absent from circulating rabbit monocytes.[4,36] It is interesting to speculate that the presence of myeloperoxidase in the Kupffer cells of some species but not others may contribute to the differential susceptibility of various species to liver damage.

An important question raised by our observations is the cellular source of myeloperoxidase. Intracellular myeloperoxidase might arise from enzyme present in storage granules or in phagolysosomes or from secreted myeloperoxidase that has bound to mannose receptors and been internalized.[37] Uptake of myeloperoxidase by macrophage mannose receptors is supported by the observation that levels of myeloperoxidase are lower in peripheral blood than in portal blood, suggesting clearance of circulating myeloperoxidase by the liver.[38] However, it is also possible that cytokines or growth factors induce myeloperoxidase gene expression in Kupffer cells or cause monocytes to continue to express the enzyme as they differentiate into macrophages. Indeed, we have recently shown that granulocyte-macrophage colony-stimulating factor causes cultured human monocytes to continue to express functional myeloperoxidase as they differentiate into macrophages.[11] The presence of myeloperoxidase in liver macrophages may increase their capacity for cytotoxicity, because internalization of myeloperoxidase has been shown to enhance the ability of macrophages to kill microorganisms.[39,40]

The ability of liver macrophages to use myeloperoxidase is demonstrated by the finding of HOCl-modified proteins in acute and chronic liver pathology. Although myeloperoxidase is present in Kupffer cells in both nondiseased and diseased livers, reactivity with HOP-1, the monoclonal antibody that specifically recognizes HOCl-modified proteins, was observed in close association with macrophages only in diseased liver, suggesting that myeloperoxidase activity arising from macrophages is enhanced during liver injury. It is important to point out, however, that because the liver sections we examined were derived primarily from explants, the patterns of im-

munostaining for HOCl-modified proteins that we observed may not be characteristic of less severe forms of liver injury.

In neutrophils and monocytes, myeloperoxidase is stored in the azurophilic granules.[4,6] Depending on the agonist for phagocyte activation, it can be secreted into intracellular phagolysosomes or discharged into the extracellular milieu.[41] The two distinct patterns of immunostaining for HOCl-modified proteins that we observed seem to indicate that hepatic macrophages also mobilize myeloperoxidase by both intra- and extracellular routes. In cases of acute, severe liver injury, HOP-1 reactivity was most prominent in necrotic areas infiltrated by macrophages. This pattern is consistent with HOCl production in the phagolysosomes of macrophages that have ingested necrotic debris. The pattern of HOP-1 reactivity was strikingly different in cases of chronic liver disease with cirrhosis in which HOCl-modified proteins were localized to hepatocytes adjacent to fibrous septa. HOP-1 staining of cells and extracellular matrix near myeloperoxidase-containing cells has previously been observed in atherosclerotic lesions and in diseased glomeruli and is presumed to result from diffusion of HOCl from its site of generation.[13,24] The present observation suggests that myeloperoxidase contained in septal macrophages had been enzymatically active in the extracellular environment.

Myeloperoxidase could contribute to liver disease in a number of ways. The HOCl it generates is a potent oxidant that directly chlorinates and oxygenates a wide range of nucleophilic moieties *in vitro*.[4,12,14–18] The enzyme also inactivates protease inhibitors and converts zymogen precursors of proteases into their active forms[19,20] and has been shown to exert damaging effects on components of the extracellular matrix.[42,43] Myeloperoxidase may therefore promote degradation of the normal hepatic extracellular matrix. Alterations in the extracellular matrix have been linked to the activation of hepatic stellate cells, which is a critical step in the development of hepatic fibrosis.[44] Furthermore, reactive species generated by myeloperoxidase may in turn peroxidize lipids, thereby enhancing collagen production by activated stellate cells.[45]

The progression of chronic liver disease to fibrosis and ultimately to cirrhosis has been linked to oxidative damage to lipids and proteins.[46] In most cases, however, the source of oxidants has not been clearly identified. In acute inflammatory processes, the generation of reactive oxygen species during the respiratory burst of neutrophils, monocytes, and macrophages is one potential source. It is noteworthy, however, that with the exceptions of alcoholic hepatitis and nonalcoholic steatohepatitis, neutrophilic infiltrates are not characteristic of chronic liver disease. Although Kupffer cells have been implicated in the pathogenesis of liver injury from a variety of causes, these macrophages have been shown to produce less superoxide on stimulation than neutrophils or other macrophages.[47] Moreover, superoxide is chemically a reducing agent and is not highly reactive with biomolecules.[3,5] Its dismutation product, H_2O_2, is a relatively weak oxidant that is much less bactericidal than HOCl.[3,5] It is therefore unclear whether reactive oxygen species derived from the respiratory burst of Kupffer cells adequately explain the oxidative damage observed in chronic liver disease. Our finding that human Kupffer cells express myeloperoxidase suggests a more likely oxidant because these cells may have the capacity to convert H_2O_2 to HOCl. Thus, Kupffer cell myeloperoxidase may be an important source of oxidative damage during chronic liver injury.

In summary, our study has shown by Western blot and by immunohistochemical studies that human Kupffer cells contain myeloperoxidase, and that this enzyme's characteristic oxidation products are detectable in acute and chronic liver disease. These observations point to a novel oxidative pathway by which Kupffer cells could participate in liver injury. These findings may have therapeutic implications because biochemical defenses against the products of myeloperoxidase differ from those that prevent damage by other reactive species.[48]

Acknowledgments

We thank Dr. Brent Tetri for providing human liver tissue, Dr. Ernst Malle for providing the antibody to HOCl-modified proteins, Jean Ross for her expertise in confocal microscopy, Raleigh Kladney for help with the figures, and Vanita Talkad for expert technical assistance.

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